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Role of GLP-1 receptor agonists in endothelial function

AKADEMISK AVHANDLING

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ABSTRACT

BACKGROUND

The leading cause of death for patients suffering from type 2 diabetes is macrovascular disease. Endothelial dysfunction is one of the earliest events identified in the pathogenesis of atherosclerosis. Hence, there is a need for finding glucose-lowering agents that cause direct positive effects on vasculature in diabetic patients. The aim of this work was to evaluate the putative role and potential effects of incretins *i.e.* GLP-1 and exendin-4 on the vasculature and to elucidate the mechanisms behind these effects.

STUDY I

We investigated whether incretins influence proliferation of human coronary artery endothelial cells (HCAECs) *in vitro* and studied the molecular mechanisms behind such effects. Exendin-4, GLP-1 (7-36) and GLP-1 (9-36) elicited dose-dependent increases in DNA synthesis and increased cell number. This mitogenic effect was associated with increased eNOS and Akt activity, which along with the augmented cell proliferation were blocked by PKA-, PI3K-, Akt- and eNOS-inhibitors and by a GLP-1 receptor antagonist, exendin (9-39).

STUDY II

We studied the role of exendin-4 on apoptosis of HCAECs under lipotoxic conditions *in vitro*. Palmitate provoked apoptosis, an effect that was inhibited by exendin-4 or GLP-1 (7-36). In contrast, palmitate-induced apoptosis was not affected by GLP-1 (9-36). Palmitate alone resulted in increased eNOS, p-38 MAPK and JNK phosphorylation, which were neutralized by exendin-4. The protective effect of exendin-4 on apoptosis was prevented after treatment of the cells with specific inhibitors for PKA, PI3K, eNOS, p38 MAPK or for JNK. The effect of exendin-4 on lipoapoptosis was blocked by the GLP-1 receptor antagonist, exendin (9-39).

STUDY III

In this study, we investigated the long-term *in vitro* effect of palmitate or high glucose, and the role of exendin-4, on gene expression in HCAECs. Our data show that the expression of eNOS was up-regulated by exendin-4 in the presence of either palmitate or high glucose, as demonstrated by both microarray and Western blotting analyses. However, microarray analysis showed a suppressed eNOS expression by palmitate, which was not observed in Western blot.

The expression of tyrosine kinase receptor Tie-2 and its ligand Ang-1 was up-regulated in the presence of exendin-4. Moreover, exendin-4 increased the expression of tissue plasminogen activator (TPA) and cell adhesion molecules involved in angiogenesis, such as platelet endothelial cell adhesion molecule (PECAM), cadherin-5 and extracellular matrix protein fibronectin. Angiotensin I-converting enzyme (ACE) expression was up-regulated by high glucose, whereas exendin-4 inhibited expression of this gene at high glucose.

STUDY IV

The aim of this study was to investigate whether exendin-4 could protect against endothelial dysfunction induced by a triglyceride-rich fat emulsion, and if there were any differences in vasorelaxant capacity between GLP-1 (7-36), GLP-1 (9-36) and exendin-4 in rat femoral arterial rings from non-diabetic rats *ex vivo*. Exendin-4 did not protect against lipotoxicity, whereas GLP-1 (7-36) and GLP-1 (9-36) exerted vasorelaxation.

CONCLUSIONS

GLP-1 receptor agonists stimulate the proliferation of HCAECs, protect them from lipoapoptosis and improve endothelial function in part through regulating expression of genes involved in angiogenesis, inflammation and thrombogenesis by reversing glucolipotoxic gene regulation. Improvement of endothelial dysfunction may translate into beneficial effects on many cardiovascular risk factors and may thus have important clinical implications in preventing and treating macroangiopathy in type 2 diabetes.